Early reprogramming regulators identified by prospective isolation and mass cytometry.

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Public Summary:

Scientific Abstract:
In the context of most induced pluripotent stem (iPS) cell reprogramming methods, heterogeneous populations of non-productive and staggered productive intermediates arise at different reprogramming time points. Despite recent reports claiming substantially increased reprogramming efficiencies using genetically modified donor cells, prospectively isolating distinct reprogramming intermediates remains an important goal to decipher reprogramming mechanisms. Previous attempts to identify surface markers of intermediate cell populations were based on the assumption that, during reprogramming, cells progressively lose donor cell identity and gradually acquire iPS cell properties. Here we report that iPS cell and epithelial markers, such as SSEA1 and EpCAM, respectively, are not predictive of reprogramming during early phases. Instead, in a systematic functional surface marker screen, we find that early reprogramming-prone cells express a unique set of surface markers, including CD73, CD49d and CD200, that are absent in both fibroblasts and iPS cells. Single-cell mass cytometry and prospective isolation show that these distinct intermediates are transient and bridge the gap between donor cell silencing and pluripotency marker acquisition during the early, presumably stochastic, reprogramming phase. Expression profiling reveals early upregulation of the transcriptional regulators Nr0b1 and Etv5 in this reprogramming state, preceding activation of key pluripotency regulators such as Rex1 (also known as Zfp42), Dppa2, Nanog and Sox2. Both factors are required for the generation of the early intermediate state and fully reprogrammed iPS cells, and thus represent some of the earliest known regulators of iPS cell induction. Our study deconvolutes the first steps in a hierarchical series of events that lead to pluripotency acquisition.